ISSN 2598 0580
Bioscientia Medicina Volume 3, Issue 2, Page No: 29-37
Available online : www.bioscmed.com
Bio Sc Med 3(2) :29-37

Antibacterial Efficacy of *Aloe vera* Sap Against *Staphylococcus aureus* and *Escherichia coli*

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Received : March 20th 2019
Accepted : May 20th 2019

Abstract

Introduction

Aloe *vera* is a plant that has been used as an alternative drug. This plant contains various compounds, like anthraquinone, saponin, flavonoid, alkaloid, and tannin that has an antibacterial effect against *Staphylococcus aureus* and *Escherichia coli.* Both of it were responsible for the infection incident. This study aims to determine the efficacy of *Aloe vera* sap as an antibacterial against *Staphylococcus aureus* and *Escherichia coli*.

Methods

An experimental study, in vitro using post-test only control group design, has been done at laboratory of Medical Faculty of Sriwijaya University, by examining the antibacterial activity of *Aloe vera* sap in five different concentration (5%, 10%, 20%, 40%, dan 80%) using well diffusion and solid dillusion method to determine the minimum bactericidal concentration (MBC). And then continued with the phytocemical screening to determine the compound inside the *Aloe vera* sap.

Results

Aloe vera sap were able to kill *Staphylococcus aureus* at 5% and *Eshcerichia coli* at 80%. Compatibility test showed that *Aloe vera* sap with concentration of 10%, 20%, 40%, and 80% are compatible with amoxicillin, therefore 80% is compatible with cefotaxime. This ability due to the compound that it contains, which is alkaloid, flavonoid, tannin, quinone, and saponin.

Conclusion

Aloe vera sap is effective as an antibacterial against *Staphylococcus aureus* and *Escherichia coli.*

Keyword: *Aloe vera* sap, antibacterial, efficacy, *Staphylococcus aureus*, *Escherichia coli*.

Introduction

Infectious disesase is one of the most common health problem that led to morbidity and mortality around the world. Example of bacteria that could cause infection are *Staphylococcus aureus* and *Escherichia coli*[1]. *Staphylococcus aureus,* a gram-positive, grape-shaped bacteria, could causes oportunistic infection, such as food poisoning, abscess, septicemia, HAIs (Healthcare Associated Infections), and even toxic shock syndrome[2,3]. This bacteria is found approximately in around 30% of all people around the world[4].

In addition to *Staphylococcus aureus, Escherichia coli* is also a bacteria that plays major role in infection disease. *Escherichia coli,* a rod-shaped gram-negative bacteria that has LPS on its cell wall[3]. This bacteria mostly live as a normal flora of teh colon, however it can also act as an oportunistic pathogen that could cause fever, diarrhea, vomiting, bloody diarrhea, and also HAIs[2]. *Escherichia coli* is one of the commonly encountered bacteria (34,3%) in RSUP dr. Mohammad Hoesin Palembang[5].

Antimicrobial agent administration such as antibiotic is one of strategy to overcome infectious problem. However, irrational use can cause antibiotic resistance[6], this will led to a high morbidity and mortality, if the treatment failed then the patient may become a carrier to resistant bacteria, and increase the treatment cost[7]. There was an increase of 50% in resistance of *Escherichia coli, Klebsiella pneumonia,* and *Staphylococcus aureus* to commonly used antibiotics. This highlight the need for further research regarding the use of medicinal plants as alternative antibiotics.

Aloe vera is one of a plant that is widely used as a medicinal plant. *Aloe vera* has been used as an anti-inflamatory, skin-protector, antiviral, and even antibacterial[8]. Sap is one part of *Aloe vera* that has an antibacterial effect. Even so, this effect is rarely explored and instead sap is commonly left as waste. This antibacterial effect derived from its active ingridients, such as alkaloid, flavonoid, anthraquinone, saponin, tannin, and steroid[9]. *Aloe vera* sap has better antibacterial activity compared to gel when tested to *Staphylococcus aureus* and *Escherichia coli*[10].

Along with the increasing incidence of antibacterial resistance, research is needed to look for alternative antibacterial sources such as those from plants, for example *Aloe vera*. *Aloe vera* contains several active compounds that has an antibacterial effect so that it could inhibit *Staphylococcus aureus* and *Escherichia coli*. Therefore, this research was conducted with the tittle of Antibacterial Efficacy of *Aloe vera* Sap Against *Staphylococcus aureus* and *Escherichia coli.*

Methods

An experimental study, in vitro using post-test only control group design, was conducted on August 2018 – January 2019 at Microbiology Laboratory and Biochemistry Laboratory, Faculty of Medicine, Universitas Sriwijaya, Palembang.

Preparation of *Aloe vera* sap

Aloe vera sap was obtained by cutting the leaves and collecting the yellow exudate into container. Then the sap was made into five different concentration, 5%, 10%, 20%, 40%, and 80% by adding aquadest as a solvent.

Phytocemical analysis

The presence of active compounds, such as alkaloid, flavonoid, tannin, saponin, and anthraquinone were analised qualitatively[11].

Determination of minimum bactericidal concentration

Minimum bactericidal concentration was determined by using solid dilution method. Serial dilution was carried out on microwell, by inserting 200 µl of 80% aloe vera sap which had been prepared with Mueller-Hinton broth in well E, then serially moving 100 µl to a lower concentration well which previously contained 100 µl Mueller-Hinton broth, until the well with the lowest concentration, 100 µl is taken and then discarded. 10 µl of bacterial suspention was added, and then incubated for 24 hours at 37 ºC. After that, one ose was taken and planted on Mueller-Hinton agar media and then incubated again. The lowest concentration where the bacteria doesn't grow, determined as minimum bactericidal concentration.

Agar well diffusion antibacterial activities

Bacterial suspention was inoculated into the Mueller-Hinton agar by using cotton swab. Between wells, a distance of 24 mm was given to prevent overlapping. The wells were filled with 40 µl of sap and control group. Inhibition zone diameter was calculated by measuring the clear zone diameter around the well in mm, after the incubation for 16-18 hours at 35 ºC.

Data analysis

Statistical analysis was carried out uing SPSS software. Data analysis was done with Kruskall-Wallis test and were followed by post-hoc test bonferroni correction.

Results

Phytochemical analysis

A total of five *Aloe vera* leaves (950 gr) is cut and produce 4 ml of sap. The results of the phytochemical test of *Aloe vera* sap shows some active compounds contained as listed in Table I.

Table 1. Result of phytochemical analysis of *Aloe vera* sap.

Compound	Method	Finding	Result
Alkaloid	Dragendorff.	Orange on filter paper.	(+)
		Red sediment.	(+)
	Mayer	No change	(-)
Flavonoid	+ HCl and amyl alcohol.	Yellow.	(+)
Saponin	Shaking and +HCl.	Forming a stable soap-like foam even after adding HCl.	(+)
Tannin	+ $FeCl_3$.	Green.	(+)
Quinone	+ NaOH.	Red.	(+)
Phenolic compound	+ $FeCl_3$.	Black.	(+)

Note: (+) Presence. (-) Absence

Determination of minimum bactericidal concentration

Data in table 2 shows that *Staphylococcus aureus* growth began to stop at the concentration of 5%, while *Eshcerichia coli* stop at 80%. This concentration is then determined as minimum bactericidal concentration.

Table 2. Minimum bactericidal concentration of *Aloe vera* sap against *Staphylococcus aureus* and *Escherichia coli*

	Concentration	*Staphylococcus aureus*	*Escherichia coli*
***Aloe vera* sap**	5%	-	+
	10%	-	+
	20%	-	+
	40%	-	+
	80%	-	-
Amoxicillin		-	-
Cefotaxime		+	+
Akuades		+	+

(+) Presence of bacterial growth, (-) Absence of bacterial growth.

Agar well diffusion antibacterial activities

Data in table 3 shows that different concentration of *Aloe vera* sap forms different inhibition diameter too. *Aloe vera* sap has an antibacterial activity against *Staphylococcus aureus*, with the largest diameter formed by concentration of 80% (17,13 ± 0,54), while the smallest was formed by 10% (8,13 ± 0,45). The inhibition zone diameter increases along with the increasing of concentration. However, *Escherichia coli* were resistant to even the largest concentration.

Then to see the difference of inhibition zone diameter between *Aloe vera* sap and control group, statistical test with Kruskall-Wallis were carried out, after obtaining a significant p value, the analysis were continued by using post-hoc to see the compatibility.

Table 3. Inhibition zone diameter of *Aloe vera* sap against *Staphylococcus aureus* and *Escherichia coli*

	Concentration	Inhibition Zona Diameter (mm)				Average ± SEM
		I	II	III	IV	
Staphylococcus aureus						
Aloe vera sap	5%	0	4	2,75	0	1,69 ± 0,46[a]
	10%	6,25	10,5	8	7,75	8,13 ± 0,45[b]
	20%	14	16,75	17,25	16,75	16,19 ± 0,37[b]
	40%	15	20,5	14,75	17,5	16,94 ± 0,62[b]
	80%	14,75	20	16	17,75	17,13 ± 0,54[b]
Amoxiciline		12,5	17,75	14,5	14,25	14,75 ± 0,51
Aquadest		0	0	0	0	0 ± 0[a]
Escherichia coli						
Aloe vera sap	5%	0	0	0	0	0 ± 0[c]
	10%	0	0	0	0	0 ± 0[c]
	20%	0	0	0	0	0 ± 0[c]
	40%	2	1,75	0	0	0,94 ± 0,25[c]
	80%	4,75	5,25	0	5,75	3,94 ± 0,60[d]
Cefotaxime		30,25	30,75	32,25	35	32,06 ± 0,57
Aquadest		0	0	0	0	0 ± 0[c]

[a] *p value* of post-hoc analysis of bonferroni correction p<0,05 compared to amoxicillin.
[b] *p value* of post-hoc analysis of bonferroni correction p>0,05 compared to amoxicillin.
[c] *p value* of post-hoc analysis of bonferroni correction p<0,05 compared to cefotaxime.
[d] *p value* of post-hoc analysis of bonferroni correction p>0,05 compared to cefotaxime.

Based on post-hoc analysis, the compatibility of effectiveness was seen between groups of 10%, 20%, 40%, and 80% with the amoxicillin group. This shows that *Aloe vera* sap with a concentration of 10%, 20%, 40%, and 80% were effective as an antibacterial against *Staphylococcus aureus.* Meanwhile, the compatibility of effectiveness was seen between group of 80% with the cefotaxime group. This shows that *Aloe vera* sap with a concentration of 80% were effective as an antibacterial against *Escherichia coli.*

Discussion

Aloe vera sap has been tested and proven to contain alkaloid, flavonoid, saponin, tannin, quinone and phenolic compound. These compounds has an antibacterial effect with various mechanisms. Alkaloid are heterocyclic nitrogen compound that usually found in nature[12]. It has been proposed that the alkaloid act by interfering nucleic acid synthesis by inhibiting dihydrofolate reductase, and disrupt the outer membrane and cytoplasmic layer that will led into a leakage of cytoplasmic content[13].

Flavonoid are hydroxylated phenolic substances with C6-C3 unit linked to an aromatic ring. Flavonoid act by interfering nucleic acid synthesis, forming bond with extracellular protein and cell wall, and disrupts the membrane[14].

Saponin are detergent-like compound that contain triterpenoid glicoside[15]. This compound acts as an antibacterial by forming a bond with porin and then disrupt it, inactivating the enzyme, and denaturating protein[16].

Tannin is a group of a polymeric phenolic substance that are found in almost all part of the plant[14]. Tanin acts by inhibiting extracellular enzyme, precipitating protein, and also has a target on the polypeptide so that it can interfere the cell wall synthesis[17].

Quinone are aromatic rings with two ketone substitutions[14]. Anthraquinone is an example of this group that contained highly on *Aloe vera* sap. This compound acts by inhibiting protein synthesis by blocking where the aminoacylated tRNA enters (ribosomal A site) .

Minimum bactericidal inhibition test was carried out to determine minimum concentration that can still kill a microorganism. This test was done by using solid dilution method. The results of this study showed that the smallest concentration of *Aloe vera* sap that can still kill *Staphylococcus aureus* was 5% and *Escherichia coli* was 80%. This findings were in agreement with Tambekar, Khante, and Dhaikar[9] who stated that the *Aloe vera* sap has an antibacterial activity against gram-positive and gram-negative bacteria. This effect due to active compound that *Aloe vera* sap contains.

Based on the result of agar well diffusion method, *Aloe vera* sap was proven to have an inhibitory effect against *Staphylococcus aureus*, this finding were corresponding with Abakar, Bakhiet, and Abadi[18]. However, *Escherichia coli* were resistant to even the largest concentration. The present findings were in disagreement with those of Kaithwas *et al*.[10] who stated that *Aloe vera* sap was effective as antibacterial against *Escherichia coli.* This differences were due to different concentration used in both studies, *Aloe vera* sap with concentration at 100% were used in the previous study, while 80% were the largest concentration in this study.

Also the finding on solid dilution method were contraindication with agar well diffution method. There were factors that affect antibacterial activity, such as the level of concentration and dosage, the inoculum density, the thickness of media agar, the incubation temperature, or the microorganism factor itself[19]. In this study there were differences in dosage of *Aloe vera* sap between solid dilution method and agar well diffution mehod. In agar well diffution method, a well that can only contains 40 µl were used, while in solid dilution method, a microwell that can contains 100 µl were used. Furthermore, in agar well diffution method *Aloe vera* sap doesnt directly in contact with bacteria, while in solid dilution method *Aloe vera* sap were directly in contact with the bacteria. This will then cause differences in results between diffution and dilution method.

Based on table 3, there were differences of effectivity against *Staphylococcus aureus* and *Escherichia coli* that representing gram-positive and gram-negative bacteria. This differences were due to the number of cell wall layer. *Staphylococcus aureus* has a simpler wall structure than *Escherichia coli* that made active compound go through inside the cell easier. Meanwhile, gram-negative bacteria cell wall was composed by three layer, outer membrane, lipoprotein, and peptydoglycan that acts as a barrier[20]. This structure difference causes sensitivity differences of *Aloe vera* sap against gram-positive and gram-negative bacteria.

Conclusion

The present study conclude that the *Aloe vera* sap were effective as an antibacterial against *Staphylococcus aureus* with the minimum bactericidal concentration at 5% and *Escherichia coli* with the minimum bactericidal concentration at 80%. Statistically *Aloe vera* sap with a concentration of 10%, 20%, 40%, and 80% compatible with amoxicillin, and 80% with cefotaxime. This effect was due to alkaloid, flavonoid, saponin, tannin, and quinone in *Aloe vera* sap.

Acknowledgment

The author thanks Microbiology Laboratory and Biochemical and Medical Chemical Laboratory Universitas Sriwijaya for providing assistance in instrument for this research.

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